REMARKS/ARGUMENTS

Claims 58-62 are pending in this application.

Applicants thank the Examiner for entering the after-final amendment filed July 28, 2004, and the preliminary amendment filed May 20, 2005.

I. Claim Rejections Under 35 U.S.C. §§101 and 112, First Paragraph

Claims 58-62 remain rejected under 35 U.S.C. §101 allegedly "because the claimed invention is not supported by either a credible, specific and substantial asserted utility or a well established utility." (Page 2 of the instant Office Action).

Claims 58-62 further remain rejected under 35 U.S.C. §112, first paragraph, allegedly "since the claimed invention is not supported by either a credible, specific and substantial asserted utility or a well established utility ..., one skilled in the art would not know how to use the claimed invention." (Pages 2-3 of the instant Office Action).

For the reasons outlined below, Applicants respectfully disagree and traverse the rejection. With respect to Claims 58-62, Applicants submit that not only has the Patent Office not established a *prima facie* case for lack of utility and enablement, but that the PRO351 polypeptide and the claimed antibodies that bind it possess a credible, specific and substantial asserted utility and are fully enabled.

First of all, Applicants respectfully maintain the position that the specification discloses at least one credible, substantial and specific asserted utility for the PRO351 polypeptide and the claimed antibodies that bind it for the reasons previously set forth in Applicants' responses filed on April 29, 2004, July 28, 2004, and May 20, 2005.

Furthermore, as first discussed in Applicants' Response of April 29, 2004, Applicants rely on the gene amplification data for patentable utility of the PRO351 polypeptide and the claimed antibodies that bind it, and the gene amplification data for the gene encoding the PRO351 polypeptide is clearly disclosed in the instant specification under Example 114. As previously discussed, a ΔCt value of at least 1.0 was observed for PRO351 in at least ten of the lung tumors listed in Table 9. Table 9 teaches that the nucleic acids encoding PRO351 showed 1.02 to 1.46 ΔCt units which corresponds to $2^{1.02}$ to $2^{1.46}$ - fold amplification or 2.03 to 2.75 amplification in ten types of human primary lung tumors, LT9, LT10, LT11, LT13, LT15, LT16, LT17, LT18, LT19 and LT21. Accordingly, the present specification clearly discloses strong evidence that the

gene encoding the PRO351 polypeptide is significantly amplified in a significant number of lung tumors.

In further support, Applicants have submitted, in their Response filed May 20, 2005, a Declaration by Dr. Audrey Goddard. Applicants particularly draw the Examiner's attention to page 3 of the Goddard Declaration which clearly states that:

> It is further my considered scientific opinion that an at least **2-fold increase** in gene copy number in a tumor tissue sample relative to a normal (*i.e.*, non-tumor) sample is significant and useful in that the detected increase in gene copy number in the tumor sample relative to the normal sample serves as a basis for using relative gene copy number as quantitated by the TaqMan PCR technique as a diagnostic marker for the presence or absence of tumor in a tissue sample of unknown pathology. Accordingly, a gene identified as being amplified at least 2-fold by the quantitative TaqMan PCR assay in a tumor sample relative to a normal sample is **useful as a marker for the diagnosis of cancer**, for monitoring cancer development and/or for measuring the efficacy of cancer therapy. (Emphasis added).

As indicated above, the gene encoding the PRO351 polypeptide shows at least a two fold amplification in ten different lung tumors. In addition, the Goddard Declaration clearly establishes that the TaqMan real-time PCR method described in Example 114 has gained wide recognition for its versatility, sensitivity and accuracy, and is in extensive use for the study of gene amplification. The facts disclosed in the Declaration also confirm that based upon the gene amplification results, one of ordinary skill would find it credible that PRO351 is a diagnostic marker of lung cancer.

The Examiner notes that "Table 9 reports a comparison of lung tumor tissue samples with a pooled sample of DNA from normal cells but not matched tissue samples (i.e., normal lung epithelium tissue)" and asserts that "it is not clear if Dr. Goddard intended the phrase 'normal samples' to include unrelated tissue samples such as those used in the specification." The Examiner concludes that "the art does not consider pooled, unrelated DNA samples to be an appropriate control." (Page 5 of the instant Office Action).

Applicants respectfully submit that the negative control taught in the specification was known in the art at the time of filing, and accepted as a true negative control as demonstrated by use in peer reviewed publications. For example, in Pitti *et al.* (Exhibit F submitted with the Response filed May 20, 2005), the authors used the same quantitative TaqMan PCR assay described in the specification to study gene amplification in lung and colon cancer of DcR3, a

decoy receptor for Fas ligand. As described, Pitti *et al.* analyzed DNA copy number "in genomic DNA from 35 primary lung and colon tumours, relative to pooled genomic DNA from peripheral blood leukocytes (PBL) of 10 healthy donors." (Page 701, col. 1; emphasis added). The authors also analyzed mRNA expression of DcR3 in primary tumor tissue sections and found tumor-specific expression, confirming the finding of frequent amplification in tumors, and confirming that the pooled blood sample was a valid negative control for the gene amplification experiments. In Bieche *et al.* (Exhibit G submitted with the Response filed May 20, 2005), the authors used the quantitative TaqMan PCR assay to study gene amplification of *myc*, *ccnd1* and *erbB2* in breast tumors. As their negative control, Bieche *et al.* used normal leukocyte DNA derived from a small subset of the breast cancer patients (page 663). The authors note that "[t]he results of this study are consistent with those reported in the literature" (page 664, col. 2), thus confirming the validity of the negative control. Accordingly, the art demonstrates that pooled normal blood samples are considered to be a valid negative control for gene amplification experiments of the type described in the specification.

The Examiner further asserts that "damaged, precancerous lung epithelium is often aneuploid," and states that "[o]ne skilled in the art would not conclude that PRO351 is a diagnostic probe for lung cancer unless it is clear that PRO351 is amplified to a clearly greater extent in true lung tumor tissue relative to non-cancerous lung epithelium." (Page 4 of the instant Office Action). In support of this assertion the Examiner refers to the previously cited reference by Hittelman.

Applicants note that the title of the Hittelman paper is "Genetic Instabilities in Epithelial Tissues at Risk for Cancer." Hittelman studied lung tissue from chronic smokers, which had been exposed for years to carcinogenic tobacco smoke. As Hittelman explains, "[t]umors of the aerodigestive tract have been proposed to reflect a 'field cancerization' process whereby the whole tissue is exposed to carcinogenic insult (e.g., tobacco smoke) and is at increased risk for multistep tumor development (page 3). The detection of increases in chromosome number therefore identifies cells which have begun the first steps in this multistep progression to cancer. Even if these particular epithelial regions are not yet cancerous, their presence is strongly correlated with the development of cancer in the target tissue as a whole. Accordingly, Hittelman concludes that "**the measurement of chromosome instability in the target tissue**

will be useful in assessing cancer risk as well as response to intervention" (page 10; emphasis added).

Accordingly, Hittelman shows that an increase in chromosome number or gene amplification is associated not with normal tissues, but with cancerous, or pre-cancerous tissues, and therefore, an increase in chromosome number or gene amplification is a useful marker for a cancerous or pre-cancerous state. Detection of pre-cancerous cells or tissues is useful because, as explained by Hittelman, it allows for assessing cancer risk, as well as response to intervention. Hence, Applicants respectfully submit that whether a pre-cancerous or tumor sample were analyzed, the showing of DNA amplification of the PRO351 gene would still be significant, since it would lead to the diagnosis of either a pre-cancerous state or a cancerous state, which is the utility asserted here. Despite the Examiner's assertion that such a use "is not well-established in the prior art," it is clear, as discussed above, that the use of amplified genes as markers for assessing cancer risk is explicitly contemplated in Hittelman *et al.*

The Examiner asserts that the data shown in Table 9 does not provide a basis for utility or enablement of the claimed antibodies, because "there is no strong correlation between gene amplification and increased mRNA or protein levels." (Pages 3-4 of the instant Office Action). In support of this assertion, the Examiner refers to previously cited references by Haynes *et al.*, Pennica *et al.*, and Konopka *et al.* As previously discussed, the teachings of Pennica *et al.* and Konopka *et al.* are not directed towards genes in general but to genes within a single family and thus, these teachings cannot support a general conclusion regarding correlation between gene amplification and mRNA or protein levels.

The Examiner states that Pennica *et al.* and Konopka *et al.*, although limited to single genes or gene families, constitute evidence that "one skilled in the art cannot assume that any one gene's amplification results in protein over-expression." (Page 5 of the instant Office Action). Applicants respectfully point out that the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, Applicants submit that in order to overcome the presumption of truth that an assertion of utility by the applicant enjoys, the Examiner must establish that **it is more likely than not** that one of ordinary skill in the art would doubt the truth of the statement of utility. Therefore, it is not legally required that there be a "necessary" correlation between the

data presented and the claimed subject matter, such that the amplification of every possible gene inevitably results in protein over-expression. The law requires only that one skilled in the art should accept that such a correlation is **more likely than not to exist**. Applicants respectfully submit that when the proper evidentiary standard is applied, a correlation must be acknowledged.

Indeed, as previously discussed, Haynes *et al.* teaches that "there was a *general trend but no strong correlation* between protein [expression] and transcript levels" (Emphasis added). Haynes studied 80 yeast proteins to show that protein levels **(i.e. protein amounts)** cannot be *accurately* predicted from the level of the corresponding mRNA transcript. (See page 1863, under Section 2.1, last line, emphasis added). Nonetheless, in Figure 1, there is a positive correlation between mRNA and protein amongst *most* of the 80 yeast proteins studied. In fact, very few data points deviated or scattered away from the expected normal or showed a lack of correlation between mRNA and protein levels (*i.e.,* an increase in mRNA resulted in a decrease in protein levels).

In support of the assertion that there is "a poor correlation between mRNA expression and protein abundance," the Examiner cites additional references by Lian *et al.* and Fessler *et al.* (Page 6 of the instant Office Action). The Examiner asserts that Lian *et al.* examined mRNA versus protein levels in differentiating myeloid cells and found that there was a poor correlation between mRNA expression and protein changes. Applicants submit that Lian *et al.* only teach that protein expression may not correlate with mRNA level in differentiating myeloid cells and does not teach anything regarding such a lack of correlation for genes in general. Myeloid cell differentiation relates to hematopoiesis and is an entirely different biological process from solid tumor development because these two process involve entirely different regulatory mechanisms and molecules. Analysis of surface antigens expressed on myeloid cells of the granulocyte-monocyte-histiocyte series during differentiation in normal and malignant myelomonocytic cells is useful in identifying and classifying human leukemias and lymphomas, but cannot be used in diagnosis of any solid tumors. Therefore, even if the teaching of Lian *et al.* accurately reflects the correlation between mRNA and protein for the particular system studied, it can not apply to tumor diagnosis assays of the present application.

In addition, the authors themselves admit that there are a number of problems with the data presented in this reference. At page 520 of this article, the authors explicitly express their

concerns by stating that "[t]hese data must be considered with several caveats: membrane and other hydrophobic proteins and very basic proteins are not well displayed by the standard 2DE approach, and proteins presented at low level will be missed. In addition, to simplify MS anlysis, we used a Coomassie dye stain rather than silver to visualize proteins, and this decreased the sensitivity of detection of minor proteins." (emphasis added). It is known in the art that Coomassie dye stain is a very insensitive method of measuring protein. This suggests that the authors relied on a very insensitive measurement of the proteins studied. The conclusions based on such measurements can hardly be accurate or generally applicable.

The Examiner also asserts that Fessler *et al.*, who examined lipopoysaccharide-activated neutrophilins, "found a 'poor concordance between mRNA transcript and protein expression changes' in human cells." (Page 6 of the instant Office Action). Again, as with Lian *et al.*, Fessler *et al.* only examined the expression level of a few proteins/RNAs in response to LPS stimulation, which involves an entirely different regulatory mechanism from that involved in tumor development. Therefore, the teachings of Fessler *et al.* do not apply here. Additionally, the PTO has overlooked a number of limitations of the study by Fessler *et al.*

For example, as admitted by Fessler *et al.*, protein identification by two-dimensional PAGE is limited to well-resolved regions of the gel, may perform less well with hydrophobic and high molecular weight proteins, and tends to select for more abundant protein species (page 31301, col. 1). Harvesting of the LPS-incubated PMNs at 4 hours may have prevented detection of earlier, transient changes and may have thereby introduced artificial transcript-protein discordance. Furthermore, the post-LPS incubation, pre-two-dimensional PAGE cell washes would be expected to remove secreted proteins from further analysis. In addition, because protein binding of Coomassie Blue has a limited dynamic range and is typically not linear throughout the range of detection, image analysis of Coomassie Blue-stained protein spots should only be consider as semi-quantitative (see page 31301, col. 1).

In summary, both Fessler *et al.* and Lian *et al.* have relied on insensitive and inaccurate methods of measuring protein expression levels. The teachings of these two references can not be relied upon to establish a *prima facie* showing of lack of utility.

In contrast, Applicants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an

elevated level. First, the articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.*, (made of record in Appellants' Response filed July 28, 2004) collectively teach that in general, gene amplification increases mRNA expression. Second, the Declaration of Dr. Paul Polakis, principal investigator of the Tumor Antigen Project of Genentech, Inc., the assignee of the present application, shows that, in general, there is a correlation between mRNA levels and polypeptide levels.

The Examiner asserts that "Orntoft et al. *could only compare the levels of about 40 well-resolved and focused abundant proteins*." (Page 7 of the instant Office Action; emphasis in original). While technical considerations did prevent Orntoft *et al.* from evaluating a larger number of proteins, the ones they did look at showed a clear correlation between mRNA and protein expression levels. As Orntoft *et al.* state, "In general there was a highly significant correlation ($p<0.005$) between mRNA and protein alterations.... 26 well focused proteins whose genes had a known chromosomal location were detected in TCCs 733 and 335, and of these 19 correlated ($p<0.005$) with the mRNA changes detected using the arrays." (See page 42, column 2 to page 34, column 2). Accordingly, Orntoft *et al.* clearly support Appellants' position that proteins expressed by genes that are amplified in tumors are useful as cancer markers.

The Examiner also appears to misunderstand the data presented by Hyman *et al.* The Examiner has asserted that "of the 12,000 transcripts analyzed, a set of 270 was identified in which overexpression was attributable to gene amplification." The Examiner concludes that "[t]his proportion is 2%; the Examiner maintains that 2% does not provide a reasonable expectation that the slight amplification of PRO351 would be correlated with elevated levels of mRNA, much less protein." (Page 7 of the instant Office Action). Applicants respectfully submit that the Examiner appears to have misinterpreted the results of Hyman *et al.* Hyman *et al.* chose to do a genome-wide analysis of a large number of genes, most of which, as shown in Figure 2, were not amplified. Accordingly, the 2% number is meaningless, as the low figure mainly results from the fact that only a small percentage of genes are amplified in the first place. The significant figure is not the percentage of genes in the genome that show amplification, but the percentage of amplified genes that demonstrate increased mRNA and protein expression.

The Examiner has further asserted that the Hyman reference "found 44% of *highly* amplified genes showing overexpression at the mRNA level, and 10.5% of highly overexpressed

genes being amplified; thus, even at the level of high amplification and high overexpression, the two do not correlate." (Page 7 of the instant Office Action). Applicants submit that the 10.5% figure is not relevant to the issue at hand. One of skill in the art would understand that there can be more than one cause of overexpression. The issue is not whether overexpression is always, or even typically caused by gene amplification, but rather, whether gene amplification typically leads to overexpression.

The Examiner's assertion is not consistent with the interpretation Hyman *et al.* themselves place on their data, stating that, "The results illustrate **a considerable influence of copy number on gene expression patterns**." (page 6242. col. 1; emphasis added). In the more detailed discussion of their results, Hyman *et al.* teach that "[u]p to 44% of the highly amplified transcripts (CGH ratio, >2.5) were overexpressed (***i.e.*, belonged to the global upper 7% of expression ratios)** compared with only 6% for genes with normal copy number." (See page 6242, col. 1; emphasis added). These details make it clear that Hyman *et al.* set a highly restrictive standard for considering a gene to be overexpressed; yet almost half of all highly amplified transcripts met even this highly restrictive standard. Therefore, the analysis performed by Hyman *et al.* clearly shows that "it is more likely than not" that a gene which is amplified in tumor cells will have increased gene expression.

With regard to the correlation between mRNA expression and protein levels, Applicants previously submitted a Declaration by Dr. Polakis, principal investigator of the Tumor Antigen Project of Genentech, Inc., the assignee of the present application, to show that mRNA expression correlates well with protein levels, in general. As previously discussed, the Utility Examination Guidelines[1] state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered." Dr. Polakis' statement that "an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell" is based on factual, experimental findings, clearly set forth in the Declaration. The Examiner's suggestion that Dr. Polakis might be misrepresenting these experimental results out of an interest in the outcome of the case is inappropriate.

[1] Part IIB, 66 Fed. Reg. 1098 (2001).

Taken together, although there are some examples in the scientific art that do not fit within the central dogma of molecular biology that there is a correlation between polypeptide and mRNA levels, these instances are exceptions rather than the rule. In the majority of amplified genes, the teachings in the art, as exemplified by Orntoft *et al.*, Hyman *et al.*, Pollack *et al.*, and the Polakis Declaration, overwhelmingly show that gene amplification influences gene expression at the mRNA and protein levels. Therefore, one of skill in the art would reasonably expect in this instance, based on the amplification data for the PRO351 gene, that the PRO351 polypeptide is concomitantly overexpressed. Thus, Applicants submit that the PRO351 polypeptides and antibodies have utility in the diagnosis of cancer and based on such a utility, one of skill in the art would know exactly how to use the claimed antibodies for diagnosis of cancer.

The Examiner again cites Hu *et al.* as showing that genes displaying a 5-fold change or less in mRNA expression in tumors compared to normal showed no evidence of a correlation between altered gene expression and a known role in the disease. However, among genes with a 10-fold or more change in expression level, there was a strong and significant correlation between expression level and a published role in the disease.

Applicants respectfully submit that Hu *et al.* does not conclusively show that it is more likely than not that the gene amplification does not result in increased expression at the mRNA and polypeptide levels. Applicants respectfully submit that Hu *et al.* manipulated various aspects of the input data in order to minimize the false positives and negatives in their analysis Applicants further submit that the statistical analysis by Hu *et al.* is not a reliable standard because the frequency of citation only reflects the current research interest in a molecule but not the true biological function of the molecule. Finally, the conclusion in Hu *et al.* only applies to a specific type of breast tumor (estrogen receptor (ER)-positive breast tumor) and can not be generalized as a principle governing microarray study of breast cancer in general, let alone the various other types of cancer genes in general. In fact, even Hu *et al.* admit that "[i]t is likely that this threshold will change depending on the disease as well as the experiment. Interestingly, the observed correlation was only found among ER-positive (breast) tumors not ER-negative tumors." (See page 412, left column). Therefore, based on these findings, the authors add, "This may reflect a bias in the literature to study the more prevalent type of tumor in the population.

Furthermore, this emphasizes that caution must be taken when interpreting experiments that may contain subpopulations that behave very differently."[2]

Accordingly, Applicants respectfully submit that the Examiner has not shown a lack of correlation between microarray data and the biological significance of cancer genes.

Finally, the Examiner has asserted that "Hanna et al. supports the rejection, in that Hanna et al. show that gene amplification does not reliably correlate with protein over-expression, and thus the level of polypeptide expression must be tested empirically." (Page 8 of the Office Action mailed December 6, 2004). Applicants respectfully point out that the Examiner appears to have misread Hanna *et al.* Hanna *et al.* clearly state that gene amplification (as measured by FISH) and polypeptide expression (as measured by immunohistochemistry, IHC) are well correlated ("in general, FISH and IHC results correlate well" (Hanna *et al.* p. 1, col. 2)). It is only a subset of tumors which show discordant results. Thus Hanna *et al.* supports Appellants' position that it is more likely than not that gene amplification correlates with increased polypeptide expression.

Appellants have clearly shown that the gene encoding the PRO351 polypeptide is amplified in at least ten lung tumors. Therefore, the PRO351 gene, similar to the HER-2/neu gene disclosed in Hanna *et al.*, is a tumor associated gene. Furthermore, as discussed above, in the majority of amplified genes, the teachings in the art overwhelmingly show that gene amplification influences gene expression at the mRNA and protein levels. Therefore, one of skill in the art would reasonably expect in this instance, based on the amplification data for the PRO351 gene, that the PRO351 polypeptide is concomitantly overexpressed.

However, even if gene amplification does not result in overexpression of the gene product (*i.e.,* the protein) an analysis of the expression of the protein is useful in determining the course of treatment, as supported by the Ashkenazi Declaration and the Hanna article (submitted with Applicants' Response filed April 29, 2004). The Examiner appears to view the testing described in the Ashkenazi Declaration and the Hanna article as experiments involving further characterization of the PRO351 polypeptide itself. In fact, such testing is for the purpose of characterizing not the PRO351 polypeptide, but the tumors in which the gene encoding PRO351

[2] *Id.* (Emphasis added).

is amplified. The PRO351 polypeptide and the claimed antibodies which bind it are therefore useful in tumor categorization, the results of which become an important tool in the hands of a physician enabling the selection of a treatment modality that holds the most promise for the successful treatment of a patient.

For the reasons given above, Applicants respectfully submit that the present specification clearly describes, details and provides a patentable utility for the claimed invention. Accordingly, Applicants request the Examiner to reconsider and withdraw the rejection of Claims 58-62 under 35 U.S.C. §§101 and 112.

CONCLUSION

In conclusion, the present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited. Should there be any further issues outstanding, the Examiner is invited to contact the undersigned attorney at the telephone number shown below.

Please charge any additional fees, including fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641** (referencing Attorney's Docket No. **39780-2630 P1C11**).

Respectfully submitted,

Date: October 28, 2005

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